The Organic Chemistry of Drug Design and Drug Action

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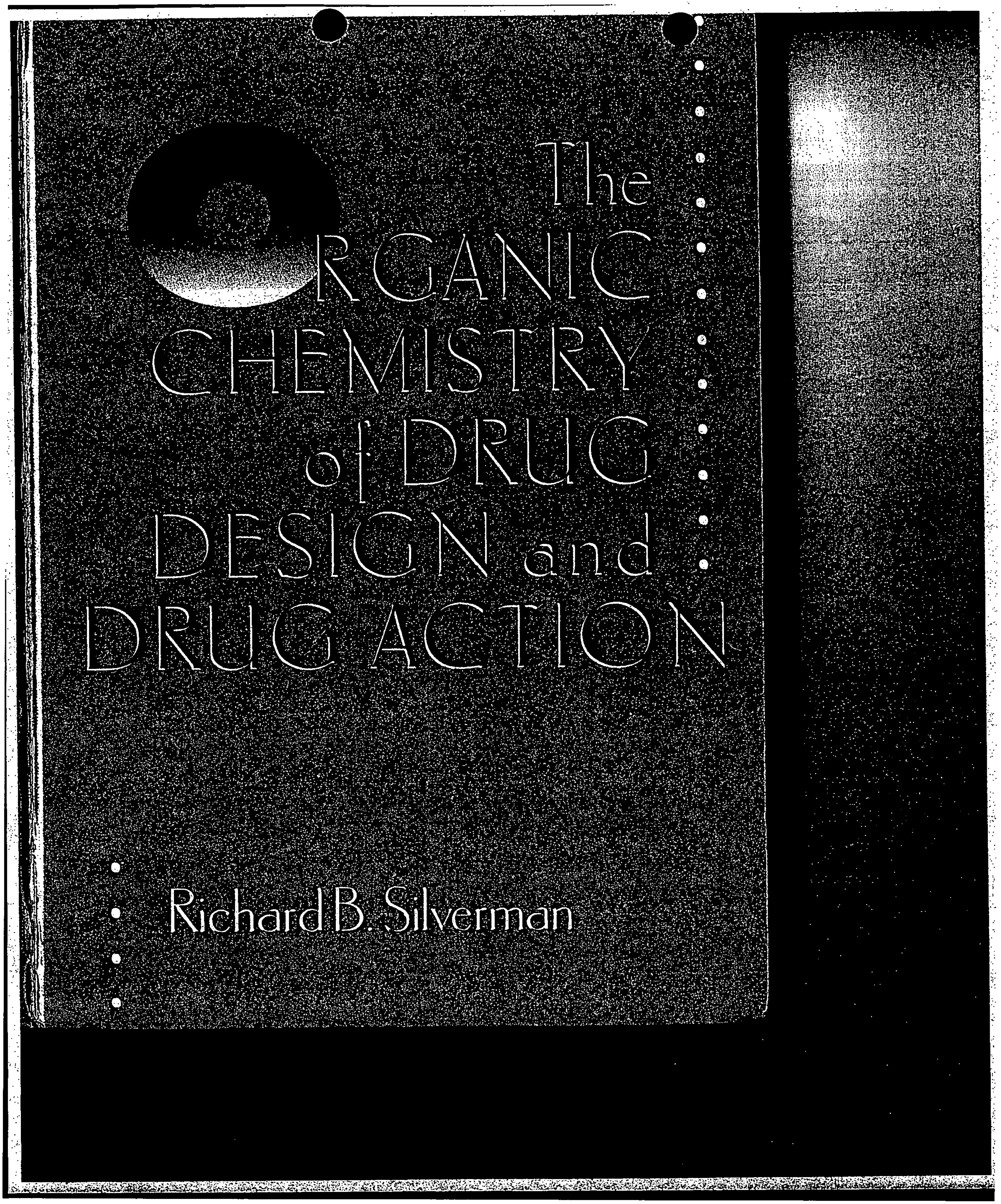

The ORGANIC CHEMISTRY of DRUG DESIGN and DRUG ACTION

Richard B. Silverman

CHAPTER 8

Prodrugs and Drug Delivery Systems

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I. Enzyme Activation of Drugs

The term *prodrug*, which was used initially by Albert,[1] refers to a pharmacologically inactive compound that is converted to an active drug by a metabolic biotransformation. A prodrug also can be activated by a nonenzymatic process such as hydrolysis, but in this case the compounds usually are inherently unstable and may cause stability problems. The prodrug-to-drug conversion can occur before absorption, during absorption, after absorption, or at a specific site in the body. In the ideal case a prodrug is converted to the drug as soon as the desired goal for designing the prodrug has been achieved. It should be noted that although the compounds discussed in this chapter are illustrative of the approaches taken for the design of prodrugs, many of them have not been approved for medical use.

A. Utility of Prodrugs

There are numerous reasons why one may wish to utilize a prodrug strategy in drug design. Specific examples of each of these categories are given in Section II,A,2.

1. Solubility

Consider an active drug that is insufficiently soluble in water so that it cannot be injected in a small dose. A water-soluble group could be attached which could be metabolically released after drug administration.

2. Absorption and Distribution

If the drug is not absorbed and transported to the target site in sufficient concentration, it can be made more water soluble or lipid soluble, depending on the desired site of action. Once absorption has occurred or when the drug is at the appropriate site of action, the water- or lipid-soluble group is removed enzymatically.

3. Site Specificity

Specificity for a particular organ or tissue can be made if there are high concentrations of or uniqueness of enzymes present at that site which can cleave the appropriate appendages from the prodrug and unmask the drug.

4. Instability

A drug may be rapidly metabolized and rendered inactive prior to when it reaches the site of action. The structure may be modified to block that metabolism until the drug is at the desired site.

5. Prolonged Release

It may be desirable to have a steady low concentration of a drug released over a long period of time. The drug may be altered so that it is metabolically converted to the active form slowly.

6. Toxicity

A drug may be toxic in its active form and would have a greater therapeutic index if it were administered in a nontoxic, inactive form that was converted to the active form only at the site of action.

7. Poor Patient Acceptability

An active drug may have an unpleasant taste or odor, produce gastric irritation, or cause pain when administered (e.g., when injected). The structure of the drug can be modified to alleviate these problems, but once administered, the altered drug can be metabolized to the active drug.

8. Formulation Problems

If the drug is a volatile liquid, it would be more desirable to prepare it in a solid form so that it could be formulated as a tablet. An inactive solid derivative could be prepared which would be converted in the body to the active drug.

B. Types of Prodrugs

There are several classifications of prodrugs. Some prodrugs are not designed as such; the biotransformations are fortuitous, and it is discovered after isolation and testing of the metabolites that activation of the drug had occurred. In most cases a specific modification in a drug has been made on the basis of known metabolic transformations. It is expected that after administration it will be appropriately metabolized to the active form. This has been termed *drug latentiation* to signify the rational design approach rather than serendipity.[2] The term drug latentiation has been refined even further by Wermuth[3] into two classes which he called carrier-linked prodrugs and bioprecursors.

A *carrier-linked prodrug* is a compound that contains an active drug linked to a carrier group that can be removed enzymatically, such as an ester which is hydrolyzed to an active carboxylic acid-containing drug. The bond to the carrier group must be labile enough to allow the active drug to be released efficiently *in vivo*, and the carrier group must be nontoxic and biologically inactive when detached from the drug. Carrier-linked prodrugs can be subdivided even further into bipartate, tripartate, and mutual prodrugs. A *bipartate prodrug* is a prodrug comprised of one carrier attached to the drug. When a carrier is connected to a linker arm which is connected to the drug, the term *tripartate prodrug* is used. A *mutual prodrug* consists of two, usually synergistic, drugs attached to each other (one drug is the carrier for the other and vice versa).

A *bioprecursor* is a compound that is metabolized by molecular modification into a new compound which is the active principle or which can be metabolized further to the active drug. For example, if the drug contains a carboxylic acid group, the bioprecursor may be a primary amine which is metabolized by oxidation to the aldehyde which is further metabolized to the carboxylic acid drug (see Section IV,B,1,e of Chapter 7). Unlike a carrier-linked prodrug, which is the active drug linked to a carrier that generally is released by a hydrolytic reaction, a bioprecursor contains a different structure that cannot be converted to the active drug by simple cleavage of a group from the prodrug.

The concept of prodrugs can be analogized to the use of protecting groups in organic synthesis.[4] If, for example, you wanted to carry out a reaction on a compound that contained a carboxylic acid group, it may be necessary first to protect the carboxylic acid as, say, an ester, so that the acidic proton of the carboxylic acid does not interfere with the desired reaction. After the desired synthetic transformation is completed, the carboxylic acid analog could be unmasked by deprotection, that is, hydrolysis of the ester (Scheme 8.1A). This is analogous to a carrier-linked prodrug; an ester functionality can be used to give the drug more desirable properties until it reaches the appropriate biological site where it is "deprotected." Another type of protecting group in organic synthesis is one which has no resemblance to the desired functional group. For example, a terminal alkene can be oxidized with ozone to an aldehyde,[5] and the aldehyde can be oxidized to a carboxylic acid with hydrogen peroxide (Scheme 8.1B). As in the case of a bioprecursor, a drastic structural change is required to unmask the desired group. Oxidation is a common metabolic biotransformation for bioprecursors.

$$\text{A.}\quad \mathrm{RCO_2H} \xrightarrow[\text{HCl}\ \Delta]{\text{EtOH}} \mathrm{RCO_2Et} \xrightarrow[\text{on R}]{\text{reaction}} \mathrm{R'CO_2Et} \xrightarrow[\Delta]{\mathrm{H_3O^+}} \mathrm{R'CO_2H}$$

$$\text{B.}\quad \mathrm{RCH{=}CH_2} \xrightarrow[\text{on R}]{\text{reaction}} \mathrm{R'CH{=}CH_2} \xrightarrow[\text{2. } \mathrm{H_2O_2}]{\text{1. } \mathrm{O_3}} \mathrm{R'CO_2H}$$

Scheme 8.1. Protecting group analogy for a prodrug.

When designing a prodrug, you should keep in mind that a particular metabolic transformation may be species specific (see Chapter 7). Therefore, a prodrug designed on the basis of rat metabolism studies may not necessarily be effective in humans.

II. Mechanisms of Prodrug Activation

A. Carrier-Linked Prodrugs

The most common reaction for activation of carrier-linked prodrugs is hydrolysis. First, we consider the general functional groups involved, then specific examples for different types of prodrugs will be given.

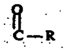
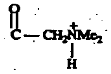
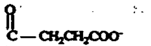
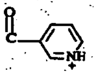
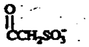
1. Carrier Linkages for Various Functional Groups

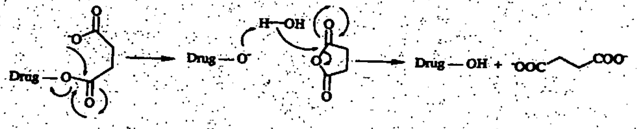
a. Alcohols and Carboxylic Acids. There are several reasons why the most common prodrug form for drugs containing alcohol or carboxylic acid functional groups is an ester. First, esterases are ubiquitous, so metabolic regeneration of the drug is a facile process. Also, it is possible to prepare ester derivatives with virtually any degree of hydrophilicity or lipophilicity. Finally, a variety of stabilities of esters can be obtained by appropriate manipulation of electronic and steric factors. Therefore, a multitude of ester prodrugs can be prepared to accommodate a wide variety of problems that require the prodrug approach.

Alcohol-containing drugs can be acylated with aliphatic or aromatic carboxylic acids to decrease water solubility (increase lipophilicity) or with carboxylic acids containing amino or additional carboxylate groups to increase water solubility (Table 8.1).[6] Conversion to phosphate or sulfate esters also increases water solubility. By using these approaches a wide range of solubilities can be achieved that will affect the absorption and distribution properties of the drug. These derivatives also can have an important effect on the dosage form, that is, whether used in tablet form or in aqueous solution. One problem with the use of this prodrug approach is that in some cases the esters are not very good substrates for the endogenous esterases, sulfatases, or phosphatases, and they may not be hydrolyzed at a rapid enough rate. When that occurs, however, a different ester can be tried. Another approach to accelerate the hydrolysis rate could be to attach electron-withdrawing groups (if a base hydrolysis mechanism is relevant) or electron-donating groups (if an acid hydrolysis mechanism is important)[7] to the carboxylate side of the ester. Succinate esters can be used to accelerate the rate of hydrolysis by intramolecular catalysis (Scheme 8.2). If the ester is too reactive, substituents can be appended that cause steric hindrance to hydrolysis. Alcohol-containing drugs also can be converted to the corresponding acetals or ketals for rapid hydrolysis in the acidic medium of the gastrointestinal tract.

Table 8.1 Ester Analogs of Alcohols as Prodrugs

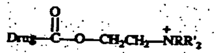
$Drug-OH \longrightarrow Drug-OX$

X	Effect on water solubility
 $\overset{\overset{O}{\|}}{C}-R$	(R = aliphatic or aromatic) decreases
 $\overset{\overset{O}{\|}}{C}-CH_2\underset{\underset{H}{\mid}}{\overset{+}{N}}Me_2$	increases ($pK_a \sim 8$)
 $\overset{\overset{O}{\|}}{C}-CH_2CH_2COO^-$	increases ($pK_a \sim 5$)
	increases ($pK_a \sim 4$)
$PO_3{}^{=}$	increases ($pK_a \sim 2$ and ~ 6)
 $\overset{\overset{O}{\|}}{C}CH_2SO_3^-$	increases ($pK_a \sim 1$)



Scheme 8.2. Intramolecular hydrolysis of succinate esters.

Carboxylic acid-containing drugs also can be esterified; the reactivity of the derivatized drug can be adjusted by appropriate structural manipulations. If a slower rate of ester hydrolysis is desired, long-chain aliphatic or sterically hindered esters can be used. If hydrolysis is too slow, addition of electron-withdrawing groups on the alcohol part of the ester can increase the rate. The pK_a of a carboxylic acid can be raised by conversion to a choline ester (**8.1**, $R = R' = Me$; $pK_a \sim 7$) or an amino ester (**8.1**, $R = H$, $R' = H$ or Me; $pK_a \sim 9$).

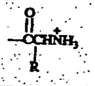
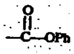
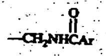

$$Drug-\overset{\overset{O}{\|}}{C}-O-CH_2CH_2-\overset{+}{N}RR'_2$$

8.1

b. Amines. N-Acylation of amines to give amide prodrugs is not commonly used, in general, because of the stability of amides toward metabolic hydrolysis. Activated amides, generally of low basicity amines, or amides of amino acids are more susceptible to enzymatic cleavage (Table 8.2). Although

Table 8.2 Prodrug Analogs of Amines

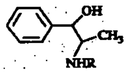
$Drug-NH_2 \longrightarrow Drug-NHX$

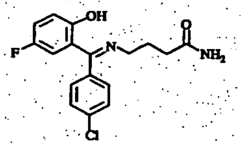
X					
$-\overset{\overset{O}{\|}}{C}R$	 $-\overset{\overset{O}{\|}}{C}\underset{\underset{R}{\mid}}{C}H\overset{+}{N}H_3$	 $-\overset{\overset{O}{\|}}{C}-OPh$	 $-CH_2NH\overset{\overset{O}{\|}}{C}Ar$	$=CHAr$	$=NAr$

carbamates in general are too stable, phenyl carbamates ($RNHCO_2Ph$) are rapidly cleaved by plasma enzymes,[8] and, therefore, they can be used as prodrugs.

The pK_a values of amines can be lowered by approximately 3 units by conversion to their *N-Mannich bases* (Table 8.2, $X = CH_2NHCOAr$). This lowers the basicity of the amine so that at physiological pH few of the prodrug molecules are protonated, thereby increasing its lipophilicity. For example, the partition coefficient (see Chapter 2, Section II,E,2,b) between octanol and phosphate buffer, pH 7.4, for the *N*-Mannich base (8.2, $R = CH_2NHCOPh$) derived from benzamide and the decongestant phenylpropanolamine (8.2, R = H) is almost 100 times greater than that for the parent amine.[9] However, the rate of hydrolysis of *N*-Mannich bases depends on the amide carrier group; salicylamide and succinimide are more susceptible to hydrolysis than is benzamide.[10]

Another approach for lowering the pK_a values of amines and, thereby, making them more lipophilic, is to convert them to imines (*Schiff bases*); however, imines often are too labile in aqueous solution. The anticonvulsant agent progabide (8.3) is a prodrug form of γ-aminobutyric acid, an important inhibitory neurotransmitter (see Chapter 5, Section V,C,3,a). The lipophilicity of 8.3 allows the compound to cross the blood–brain barrier; once inside the brain it is hydrolyzed to γ-aminobutyric acid.[11]


8.2

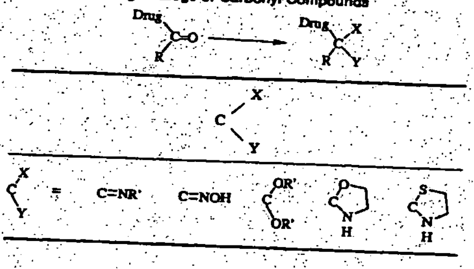

8.3

c. Carbonyl Compounds. The most important prodrug forms of aldehydes and ketones are Schiff bases, oximes, acetals (ketals), enol esters, oxazolidines, and thiazolidines (Table 8.3). A more complete review of bioreversible derivatives of the functional groups was written by Bundgaard.[6]

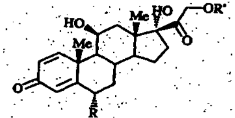
2. Examples of Carrier-Linked Bipartate Prodrugs

a. Prodrugs for Increased Water Solubility. Prednisolone (8.4; R = R′ = H) and methylprednisolone (8.4; $R = CH_3$, R′ = H) are poorly water-soluble corticosteroid drugs. In order to permit aqueous injection or ophthalmic delivery of these drugs, they must be converted to water-soluble forms such as one of the ionic esters described in Section II,A,1,a. However, there are two considerations in the choice of a solubilizing group: the ester must be stable enough in aqueous solution so that a ready-to-inject solution has a reasonably long shelf life (greater than 2 years; half-life about 13 years), but it must be hydrolyzed *in vivo* with a reasonably short half-life after administration (less than 10 min). For this optimal situation to occur the *in vivo*/*in vitro* lability ratio would have to be on the order of 10^6. This is possible when the biotransformation is enzyme catalyzed.

Table 8.3 Prodrug Analogs of Carbonyl Compounds

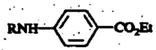


The water-soluble prodrug form of methylprednisolone that is in medical use is methylprednisolone sodium succinate (8.4, $R = CH_3$, $R' = COCH_2CH_2CO_2Na$). However, the *in vitro* stability is low; consequently, it is distributed as a lyophilized (freeze-dried) powder that must be reconstituted with water and then used within 48 hr. The lyophilization process adds to the cost of the drug and makes its use less convenient. On the basis of physical–organic chemical rationalizations, a series of more stable water-soluble methylprednisolone esters was synthesized, and several of the analogs were shown to have shelf lives in solution of greater than 2 years at room temperature.[12] Ester hydrolysis studies of these compounds in human and monkey serum indicated that derivatives having an anionic solubilizing moiety such as carboxylate or sulfonate are poorly or not hydrolyzed, but compounds with a


8.4

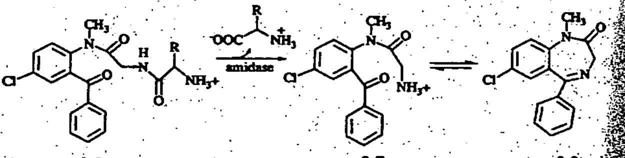
cationic (tertiary amino) solubilizing moiety are hydrolyzed rapidly by serum esterases.[13] Prednisolone phosphate (8.4; R = H, R′ = PO_3Na_2) is prescribed as a water-soluble prodrug for prednisolone that is activated *in vivo* by phosphatases.

The local anesthetic benzocaine (8.5, R = H) has been converted to water-soluble amide prodrug forms with various amino acids (8.5, R = $\overset{+}{N}H_3$-CHR′CO); amidase-catalyzed hydrolysis in human serum occurs rapidly.[14]



8.5

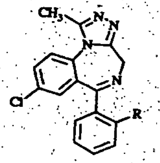
Another prodrug approach is to design acyclic derivatives that are enzymatically hydrolyzed to a product that spontaneously cyclizes to the desired drug. The benzodiazepine tranquilizer diazepam (8.8, Scheme 8.3) is very sparingly water soluble, but the open chain amino ketone coupled to an amino acid or peptide is a stable, freely water-soluble prodrug (8.6); *in vivo* peptidases hydrolyze the peptide bond, and the resulting 2-aminoacetamidobenzophenone analog (8.7) spontaneously cyclizes to give the benzodiazepine.[15,16] The rate of *in vivo* hydrolysis of the peptide bond depends on which L-amino acid is attached; peptides derived from Phe and Lys are cleaved much faster than those from Gly and Glu. The rate of cyclization depends on the substituents in the phenyl ring and on the amide nitrogen. Although the cyclization of 8.7 to 8.8 occurs with a half-life of 73 sec, that for the corresponding *N*-desmethyl analog is 15 min. As an example of how effective this approach is for increasing the water solubility, the benzodiazepine triazolam (8.9, R = Cl) has a solubility of 0.015 mg/ml at 25° C, but the corresponding open-chain glycylaminobenzophenone derivative (HCl salt) has a solubility of 109 mg/ml.[17] A similar prodrug approach was taken for the benzodiazepine alprazolam (8.9, R = H).[18]



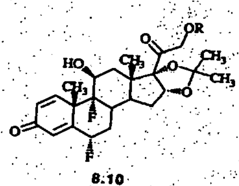
Scheme 8.3. Benzodiazepine prodrug activation.

b. Prodrugs for Improved Absorption and Distribution. The skin is designed to maintain the body fluids and prevent absorption of xenobiotics int

the general circulation. Consequently, drugs applied to the skin are poorly absorbed.[19] Even steroids have low dermal permeability, particularly if they contain hydroxyl groups which can interact with the skin or binding sites in the keratin. Corticosteroids for the topical treatment of inflammatory, allergic, and pruritic skin conditions can be made more suitable for topical absorption by esterification or acetonidation. For example, both fluocinolone acetonide (8.10, R = H) and fluocinonide (8.10, R = $COCH_3$) are prodrugs used for inflammatory and pruritic manifestations. Once absorbed through the skin an esterase releases the drug.

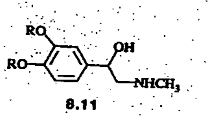


8.9



8.10

Dipivaloylepinephrine (dipivefrin; 8.11, R = Me_3CCO), a prodrug for the antiglaucoma drug epinephrine (8.11, R = H), is better able to penetrate the cornea than is epinephrine. The cornea and aqueous humor have significant esterase activity.[20]

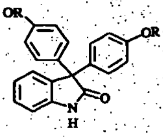


8.11

c. Prodrugs for Site Specificity. The targeting of drugs for a specific site in the body by conversion to a prodrug is plausible when the physicochemical properties of the parent drug and prodrug are optimal for the target site. It should be kept in mind, however, that when the lipophilicity of a drug is increased, it will improve passive transport of the drug nonspecifically to all tissues.

Oxyphenisatin (8.12, R = H) is a bowel sterilant that is active only when administered rectally. However, when the hydroxyl groups are acetylated (8.12, R = Ac), the prodrug can be administered orally, and it is hydrolyzed at the site of action in the intestines to oxyphenisatin.

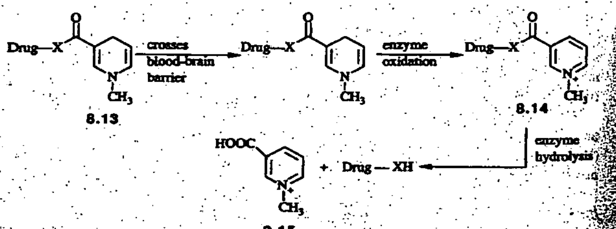
One important membrane that often is targeted for drug delivery is the *blood–brain barrier*, a unique lipidlike protective barrier that prevents hydrophilic compounds from entering the brain unless they are actively transported.[21] The blood–brain barrier also contains active enzyme systems to



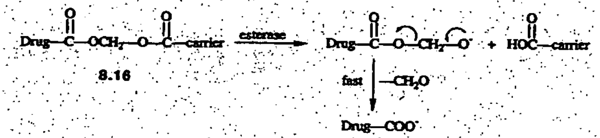
8.12

protect the central nervous system even further. Consequently, molecular size and lipophilicity are often necessary, not sufficient, criteria for gaining entry into the brain.[22] Also, once the drug has entered the brain, it must be modified so that it does not escape.

Bodor and co-workers have devised a reversible redox drug delivery system for getting drugs into the central nervous system and then, once in, preventing their efflux.[22,23] The approach is based on the attachment of a hydrophilic drug to a lipophilic carrier (a dihydropyridine, **8.13**) thereby making the bipartate prodrug overall lipophilic (Scheme 8.4). Once inside the brain, the lipophilic carrier is converted enzymatically to a highly hydrophilic species (**8.14**), which is then enzymatically hydrolyzed back to the drug and *N*-methylnicotinic acid (**8.15**) which is eliminated from the brain. The XH group on the drug is an amino, hydroxyl, or carboxyl group. When it is a carboxylic acid, the linkage is an acyloxymethyl ester (**8.16**), which decomposes by the reaction shown in Scheme 8.5. The oxidation of the dihydropyridine (**8.13**) to the pyridinium ion (**8.14**) (half-life generally 20–50 min) prevents the drug from escaping out of the brain because it becomes charged. This drives the equilibrium of the lipophilic precursor (**8.13**) throughout all of the tissues of the body to favor the brain. Any oxidation occurring outside of the brain produces a hydrophilic species that can be rapidly eliminated from the body (see Chapter 7). The released oxidized carrier (**8.15**) is relatively nontoxic and easily eliminated from the brain. Although this is a carrier-linked prodrug, it requires enzymatic oxidation to target the drug to the brain. The oxidation reaction is a bioprecursor reaction (see Section II,B,2,c).

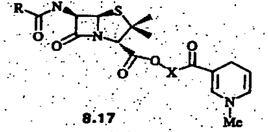


Scheme 8.4. Redox drug delivery system.



Scheme 8.5. Hydrolysis of acyloxymethyl esters.

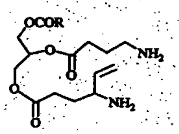
An example of this approach is the brain delivery of β-lactam antibiotics for the possible treatment of bacterial meningitis. The difficulty in purging the central nervous system of infections arises from the fact that the cerebrospinal fluid contains less than 0.1% of the number of immunocompetent leukocytes found in the blood and almost no immunoglobins; consequently, antibody generation to these foreign organisms is not significant. Since the β-lactam antibiotics are hydrophilic, they enter the brain very slowly, but they are actively transported back into the blood. Therefore, they are not as effective in the treatment of brain infections as elsewhere. Bodor and co-workers[24] prepared a variety of penicillin prodrugs attached to the dihydropyridine carrier through various linkers (**8.17**) and showed that β-lactam antibiotics could be delivered in high concentrations into the brain.



8.17

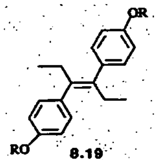
As was discussed in Section V,C,3,a of Chapter 5, increasing the brain concentration of the inhibitory neurotransmitter β-aminobutyric acid (GABA) results in anticonvulsant activity. However, GABA is too polar to cross the blood–brain barrier, so it is not an effective anticonvulsant drug. In order to increase the lipophilicity of GABA, a series of γ-aminobutyric acid and γ-aminobutyric Schiff bases were synthesized.[11] Progabide (**8.3**) emerged as an effective lipophilic analog of GABA that crosses the blood–brain barrier, releases GABA inside the brain, and shows anticonvulsant activity.[25]

Another related approach for anticonvulsant drug design was the synthesis of a glyceryl lipid (**8.18**, R = linolenoyl) containing one GABA molecule and one vigabatrin molecule, a mechanism-based inactivator of GABA aminotransferase and anticonvulsant drug (see Section V,C,3,a of Chapter 5).[26] This compound inactivates GABA aminotransferase *in vitro* only if brain esterases are added to cleave the vigabatrin from the glyceryl lipid. It also is 300 times more potent than vigabatrin, *in vivo*, presumably because of its increased ability to enter the brain.



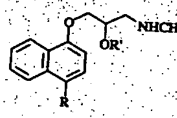
8.18

In the above examples, the lipophilicity of the drugs was increased so that they could diffuse through various membranes. Another approach for site-specific drug delivery is to design a prodrug that requires activation by an enzyme found predominantly at the desired site of action. For example, tumor cells contain a higher concentration of phosphatases and amidase than do normal cells. Consequently, a prodrug of a cytotoxic agent could be directed to tumor cells if either of these enzymes were important to the prodrug activation process. Diethylstilbestrol diphosphate (8.19, R = PO_3^{2-}) was designed for site-specific delivery of diethylstilbestrol (8.19, R = H) to prostatic carcinoma tissue.[2,27] In general, though, this tumor-selective approach has not been very successful because the appropriate prodrugs are too polar to reach the enzyme site, the relative enzymatic selectivity is insufficient, and the tumor cell perfusion rate is too poor.



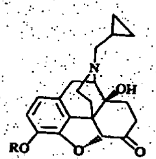
8.19

d. Prodrugs for Stability. Some prodrugs protect the drug from the first-pass effect (see Section I of Chapter 7). Propranolol (8.20, R = R′ = H) is a widely used antihypertensive drug, but because of first-pass elimination an oral dose has a much lower bioavailability than does an intravenous injection. The major metabolites (see Chapter 7) are propranolol *O*-glucuronide (8.20, R = H, OR′ = glucuronide), *p*-hydroxypropranolol (8.20, R = OH, R′ = H), and its *O*-glucuronide (8.20, R = OH, OR′ = glucuronide). The hemisuccinate ester of propranolol (8.20, R = H, R′ = $COCH_2CH_2COOH$) was prepared to block glucuronide formation; following oral administration of propranolol hemisuccinate, the plasma levels of propranolol were 8 times greater than when propranolol was used.[28]



8.20

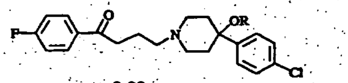
Naltrexone (8.21, R = H), used in the treatment of opioid addiction, is nonaddicting and is well absorbed from the gastrointestinal tract. However, it undergoes extensive first-pass metabolism when given orally. Ester prodrugs, namely, the anthranilate (8.21, R = CO-*o*-NO_2Ph) and the acetylsalicylate (8.21, R = CO-*o*-AcOPh), enhanced the bioavailability 45- and 28-fold, respectively, relative to 8.21 (R = H).[29]



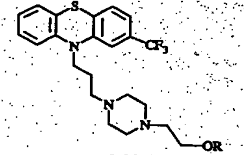
8.21

e. Prodrugs for Slow and Prolonged Release. The utility of slow and prolonged release of drugs is severalfold. (1) It reduces the number and frequency of doses required. (2) It eliminates nighttime administration of drugs. (3) Because the drug is taken less frequently, slow, prolonged release minimizes patient noncompliance. (4) When a fast released drug is taken, there is a rapid surge of the drug throughout the body. As metabolism of the drug proceeds, the concentration of the drug diminishes. A slow release drug would eliminate the peaks and valleys of fast released drugs which are a strain on cells. (5) Because a constant lower concentration of the drug is being released, it reduces the possibility of toxic levels of drugs. (6) It reduces gastrointestinal side effects. A common strategy in the design of slow release prodrugs is to make a long chain aliphatic ester because these esters hydrolyze slowly.

Prolonged release drugs are quite important in the treatment of psychoses because these patients require medication for extended periods of time and often show high patient noncompliance rates. Haloperidol (8.22, R = H) is a potent, orally active central nervous system depressant, sedative, and tranquilizer. However, peak plasma levels are observed between 2 and 6 hr after administration. Haloperidol decanoate [8.22, R = $CO(CH_2)_8CH_3$], however, is injected intramuscularly as a solution in sesame oil and its antipsychotic activity lasts for about 1 month.[30] The antipsychotic fluphenazine (8.23, R = H) also has a short duration of activity (6–8 hr). Fluphenazine enanthate [8.23, R = $CO(CH_2)_5CH_3$] and fluphenazine decanoate [8.23, R = $CO(CH_2)_8CH_3$], however, have durations of activity of about a month.[31]

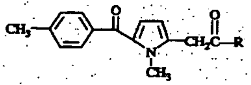


8.22



8.23

Conversion of the nonsteroidal anti-inflammatory (antiarthritis) drug tolmetin sodium (8.24, R = $O^- Na^+$) to the corresponding glycine conjugate (8.24, R = $NHCH_2COOH$) increases the potency and extends the peak concentration of tolmetin from 1 to about 9 hrs because of the slow hydrolysis of the prodrug amide linkage.[32]

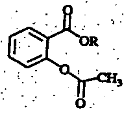


8.24

f. Prodrugs to Minimize Toxicity. The prodrugs that were designed for improved absorption (Section II,A,2,b), for site specificity (Section II,A,2,c), for stability (Section II,A,2,d), and for slow release (Section II,A,2,e) also lowered the toxicity of the drug. For example, epinephrine (8.11, R = H) (see Section II,A,2,b), used in the treatment of glaucoma, has a number of ocular and systemic side effects associated with its use. The prodrug dipivaloylepinephrine (8.11, R = Me_3CCO), has been shown to be more potent than epinephrine in dogs and rabbits and nearly as effective in humans[20] with a significantly improved toxicological profile compared with epinephrine.

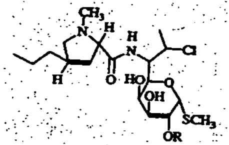
Another example of the utility of the prodrug approach to lower the toxicity of a drug can be found in the design of aspirin (8.25, R = H) analogs.[33] Side effects associated with the use of aspirin are gastric irritation and bleeding. The gastric irritation and ulcerogenicity associated with aspirin use may result from an accumulation of the acid in the gastric mucosal cells. Esterification of

aspirin (8.25, R = alkyl) and other nonsteroidal anti-inflammatory agents greatly suppresses gastric ulcerogenic activity. However, esterification also renders the acetyl ester of aspirin extremely susceptible to enzymatic hydrolysis (the $t_{1/2}$ for deacetylation of aspirin in human plasma is about 2 hrs, but that for deacetylation of aspirin esters is 1–3 min). Esters of certain N,N-disubstituted 2-hydroxyacetamides (8.25, R = $CH_2CONR_1R_2$) were found to be chemically highly stable but were hydrolyzed very rapidly by pseudocholinesterase (cholinesterase) in plasma[33]; therefore, they are well suited as aspirin prodrugs to lower the gastric irritation effects of aspirin.



8.25

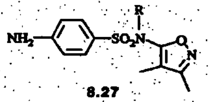
g. Prodrugs to Encourage Patient Acceptance. A fundamental tenet in medicine is that in order for a drug to be effective, the patient has to take it. Painful injections and unpleasant taste or odor are the most common reasons for the lack of patient acceptance of a drug. An excellent example of how a prodrug can increase the potential for patient acceptance is related to the antibacterial drug clindamycin (8.26, R = H). Whereas clindamycin causes pain on injection, the prodrug clindamycin phosphate (8.26, R = PO_3H_2) is well tolerated; hydrolysis of the prodrug *in vivo* occurs with a $t_{1/2}$ of approximately 10 min.[34] Also, clindamycin has a bitter taste, so it is not well accepted by children who do not take pills. However, it was found that by increasing the chain length of 2-acyl esters of clindamycin the taste improved from bitter (acetate ester) to no bitter taste (palmitate ester).[35] Of course, when dealing with young children, it is not sufficient for a drug to be just tasteless; consequently, clindamycin palmitate [8.26, R = $CO(CH_2)_{14}CH_3$] is sold for pediatric use in a cherry-flavored syrup. Bitter taste results from a compound dissolving in the saliva and interacting with a bitter taste receptor in the mouth. Esterification with long-chain fatty acids makes the drug less water soluble



8.26

and unable to dissolve in the saliva. It also may alter the interaction of the compound with the taste receptor.

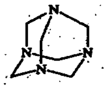
The antibacterial sulfa drug sulfisoxazole (**8.27**, R = H) also is bitter tasting, but sulfisoxazole acetyl (**8.27**, R = $COCH_3$) is tasteless. For pediatric use this drug is combined with the tasteless prodrug form of erythromycin, namely, erythromycin ethylsuccinate, in a strawberry–banana-flavored suspension (my 6-year-old loves it).



8.27

h. Prodrugs to Eliminate Formulation Problems. Formaldehyde (CH_2O) is a flammable, colorless gas with a pungent odor that is used as a disinfectant. Solutions of high concentrations of formaldehyde are toxic. Consequently, it cannot be used directly in medicine. However, the reaction of formaldehyde with ammonia produces a stable adamantane-like solid compound, methenamine (**8.28**). In media of acidic pH, methenamine hydrolyzes to formaldehyde and ammonium ions. Since the pH of urine in the bladder can be made acidic, methenamine is used as a urinary tract antiseptic.[36] To prevent hydrolysis of this prodrug in the acidic environment of the stomach, the tablets are enteric coated.

The topical fungistatic prodrug triacetin (**8.29**) owes its activity to acetic acid, the product of skin esterase hydrolysis of triacetin.



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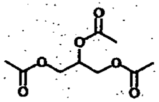


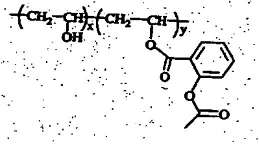
8.29

3. Macromolecular Drug Carrier Systems

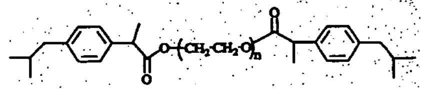
a. General Strategy. Although the prodrug approach has been very fruitful in general, there are three areas that need improvement: site specificity, protection of the drug from biodegradation, and minimization of side effects. Another carrier-linked bipartate prodrug approach that has been utilized to address these shortcomings is *macromolecular drug delivery*. This is a drug carrier system in which the drug is covalently attached to a macromolecule, such as a synthetic polymer, a glycoprotein, a lipoprotein, a lectin, a hormone, albumin, a liposome, DNA, dextran, an antibody, or a cell. Because the absorption and distribution of the drug depend on the physicochemical properties of the macromolecular carrier, not the drug, these parameters can be altered by manipulation of the properties of the carrier. This approach has the potential advantage of targeting drugs for a specific site and improving the therapeutic index by minimizing interactions with nontarget tissues (i.e., lowering the toxicity) as well as reducing premature drug metabolism and excretion. However, it has the disadvantages that the macromolecules may not be well absorbed after oral administration, requiring alternative means of administration, and may be immunogenic. Although polymer conjugates generally cannot pass through membranes, they can gain access to the interior of a cell by *pinocytosis*, the process by which the cell membrane invaginates the particle and then pinches itself off to form an intracellular vesicle which moves into the cell and eventually fuses with lysosomes. Because the breakdown of proteins and other macromolecules is believed to occur in the lysosomes,[37] and because this breakdown then liberates the drug, the design of a macromolecular drug carrier system should be a fruitful approach to deliver the drug inside a cell.

An ideal drug carrier (macromolecular or otherwise) must (1) protect the drug until it is at the site of action, (2) localize the drug at the site of action, (3) allow for release of the drug chemically or enzymatically, (4) minimize host toxicity, (5) be biodegradable, biochemically inert, and nonimmunogenic, (6) be easily prepared inexpensively, and (7) be chemically and biochemically stable in its dosage form. Some of the macromolecular drug carrier systems exert their effects while the drug is still attached to the carrier, but these are not prodrugs. Several examples of macromolecular drug carrier systems follow.

b. Synthetic Polymers. Aspirin linked to poly(vinyl alcohol) (**8.30**) was shown to have the same potency as aspirin but was less toxic. Another anti-inflammatory agent, ibuprofen (the carboxylic acid of **8.31**) was attached as a poly(oxyethylene) diester (**8.31**).[38] This macromolecular carrier system resulted in a sustained release of ibuprofen, giving prolonged anti-inflammatory activity and a higher plasma half-life relative to the free drug.

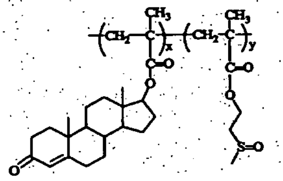


8.30

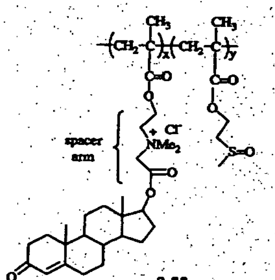


8.31

Because it is necessary for a drug to be released from the polymer backbone, steric hindrance by the polymer to chemical or enzymatic hydrolysis may cause problems. For example, when the steroid hormone testosterone is linked to poly(methacrylate) (8.32), no androgenic effect is observed, however, when a spacer arm is inserted between the polymer and the testosterone (8.33), the macromolecular drug carrier was as effective as testosterone. The 3-thiabutyl oxide chain was attached to the polymer to enhance water solubility.



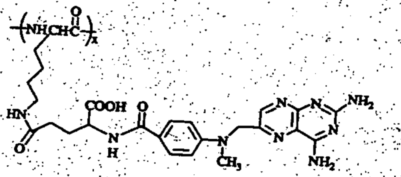
8.32



8.33

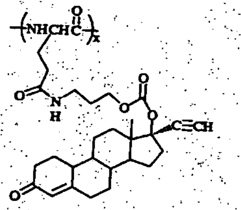
c. Poly(α-Amino Acids). The disadvantage of using synthetic polymers is that they are generally not biodegradable and can take 5–12 months to be eliminated from the body. Poly(α-amino acids) are biodegradable (at least the L-isomers are), with the rate of biodegradability depending on the choice of amino acid.

Conjugation of the antitumor drug methotrexate to poly(L-lysine) (8.34; attachment of the polymer also may be to the α-carboxyl group) markedly increased the cellular uptake of the drug and provided a new way to overcome drug resistance related to deficient drug transport.[39] As the activity of methotrexate is a function of its ability to inhibit dihydrofolate reductase (see Section III,B of Chapter 4) and 8.34 is a poor inhibitor of this enzyme *in vitro*, the methotrexate must become detached from the polymer backbone inside the cell. Furthermore, attachment of methotrexate to poly(D-lysine), which, unlike poly(L-lysine) does not undergo proteolytic digestion inside the cell, gave a conjugate devoid of activity with resistant or normal cell lines. Methotrexate attached to poly(L-lysine) also is more inhibitory to the growth of human solid tumor cell lines than to the growth of human lymphocytes; free methotrexate is equally toxic to both kinds of cells.[40]



8.34

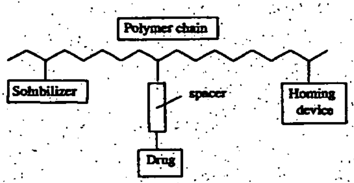
Research directed at a sustained release contraceptive resulted in the macromolecular drug delivery system 8.35.[41] The contraceptive norethindrone was attached via a 17-carbonate linkage to poly-N^5-(3-hydroxypropyl)-L-glutamine. In rats the contraceptive agent was slowly released over a 9-month period.



8.35

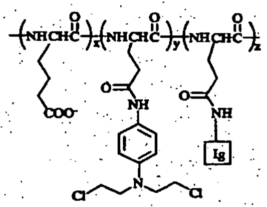
A general scheme for the design of a site-specific macromolecular drug delivery system was described by Ringsdorf[42] (8.36). A drug is attached to the polymer backbone, usually through a spacer so that it can be cleaved hydrolytically or enzymatically without steric hindrance. The desired solubility of the drug–polymer conjugate can be adjusted by attachment of an appropriate hydrophilic or hydrophobic ligand. Finally, site specificity, for example, to a particular cancer cell line, can be manipulated by attachment of a "homing device" such as an antibody raised against that cell line.

An elegant example of this approach in which a nitrogen mustard was delivered to tumor cells is shown in 8.37.[43] Poly(L-glutamate) was used as the



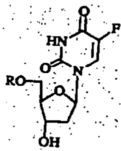
8.36

polymeric backbone so that the side-chain carboxylic acid groups could be functionalized appropriately. The water-solubilizing groups were the unsubstituted glutamate side-chain carboxylate groups, the antitumor alkylating agent (the *p*-phenylenediamine mustard) was attached to the built-in spacer arm, that is, to the glutamate side chain, and the homing device was an immunoglobulin (Ig) derived from a rabbit antiserum against mouse lymphoma cells. This macromolecular drug delivery system was much more effective than the individual components or a mixture of the components. Whereas none of the five control mice was alive and tumor free after 60 days, all five of the polymer prodrug-treated mice were. Also, the therapeutic index of *p*-phenylenediamine mustard is greatly enhanced (40-fold) when it is attached to the polymer system, because it is less toxic to normal proliferating cells. Similar results were obtained when the neutral and water-soluble polymer dextran was used.[44]

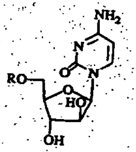


8.37

d. Other Macromolecular Supports. Because inhibitors of DNA synthesis generally are toxic to normal rapidly proliferating cells as well, a targeted macromolecular approach to the delivery of the antitumor agents floxuridine (**8.38**, R = H) and cytosine arabinoside (cytarabine; **8.39**, R = H) was taken to decrease their toxicity.[45] The drugs were conjugated to albumin because once proteins enter cells, they are rapidly broken down by lysosomal enzymes, and this would release the drugs from the albumin inside the cells. As certain neoplastic proliferating cells are highly endocytic (high protein uptake) and normal cells with high protein uptake do not proliferate, selective toxicity to neoplastic cells or to DNA viruses that replicate in cells with high protein uptake could be accomplished. Both conjugates (**8.38** and **8.39**, R = albumin–CO) were shown to inhibit the growth of *Ectromelia* virus in mouse liver, whereas the free inhibitors were ineffective. The conjugates exert their antiviral activity in liver macrophages (cells with high protein uptake), suggesting that the drugs are concentrated in these cells.

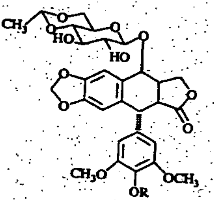


8.38



8.39

A clever strategy for the delivery of cytotoxic agents to solid tumors was devised.[46] Monoclonal antibodies raised against tumor cells are used as carriers of enzymes that are capable of converting nontoxic prodrugs of antitumor agents to active drugs. The prodrugs are converted to drugs at the desired site of action where they then can penetrate into the tumor cell and destroy it. Alkaline phosphatase (which catalyzes the hydrolysis of phosphate esters) conjugated with a monoclonal antibody to human carcinomas was injected into mice, then phosphates of etoposide (**8.40**, R = PO_3^{2-}) and other antitumor agents were administered. Pronounced antitumor activities were observed in animals treated with the antibody–enzyme conjugate prior to the phosphates. Similar results were obtained when carboxypeptidases were attached to antibodies and injected into mice prior to administration of antitumor agent pep-

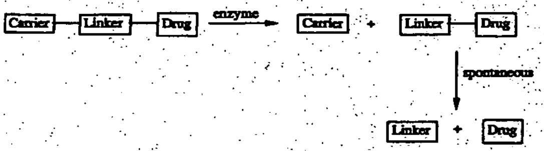


8.40

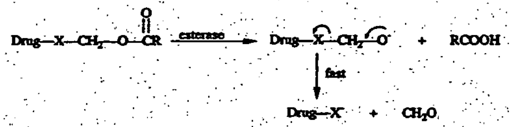
tides or when penicillin V amidase–antibody conjugates were used with amide prodrugs of antitumor agents.

4. Tripartate Prodrugs

Bipartate prodrugs may be ineffective because the prodrug linkage is too labile (e.g., certain esters) or too stable (because of steric hindrance to hydrolysis). In a tripartate prodrug the carrier is not connected directly to the drug, but rather to a linker arm which is attached to the drug (Scheme 8.6).[47] This allows for different kinds of functional groups to be incorporated for varying stabilities, and it also displaces the drug farther from the hydrolysis site, which decreases the steric interference by the carrier [as was suggested by Ringsdorf[42] for macromolecular drug delivery systems (see 8.36)]. The drug–linker connection, however, must be designed so that it cleaves spontaneously after the carrier has been detached. One approach to accomplish this has been termed the *double prodrug* or, in the case where X is COO, the *double ester* concept, generalized in Scheme 8.7[8] (X = COO, O, NH; the double ester strategy was shown earlier in Scheme 8.5).



Scheme 8.6. Tripartate prodrugs.

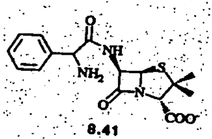


$$\mathrm{Drug{-}X{-}CH_2{-}O{-}\overset{\overset{O}{\|}}{C}R} \xrightarrow{\text{esterase}} \mathrm{Drug{-}X{-}CH_2{-}O^{-}} + \mathrm{RCOOH}$$

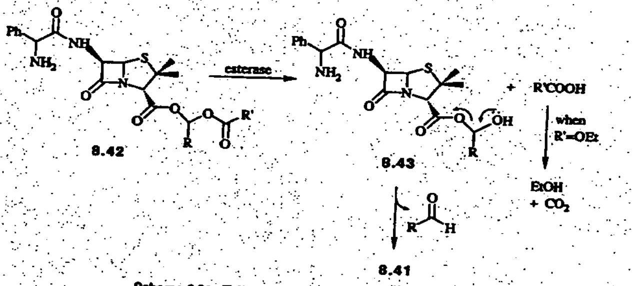
$$\xrightarrow{\text{fast}} \mathrm{Drug{-}X^{-}} + \mathrm{CH_2O}$$

Scheme 8.7. Double prodrug concept.

This strategy was employed in the design of prodrugs of ampicillin (8.41), a β-lactam antibiotic that is poorly absorbed when administered orally. As only 40% of the drug is absorbed, 2.5 times more must be administered orally than by injection. Furthermore, the nonabsorbed antibiotic may destroy important intestinal bacteria. A lipid-soluble prodrug of ampicillin would be a useful approach to increase absorption of the drug. However, although various simple alkyl and aryl esters of the thiazolidine carboxyl group are hydrolyzed rapidly to ampicillin in rodents, they are too stable in man to be therapeutically useful. This suggests that the esterases in rodents and man are different and that, most likely, steric hindrance of the ester carbonyl by the thiazolidine ring is important in the human esterase. A solution to the problem was the construction of a "double ester," an acyloxymethyl ester[48] such as 8.42 (R = CH_3, R′ = OEt; bacampicillin)[49] or 8.42 (R = H, R′ = *tert*-Bu; pivampicillin)[50] (Scheme 8.8), which would extend the terminal ester carbonyl away from the thiazolidine ring and eliminate the inherent steric hindrance with the enzyme. Hydrolysis of the terminal ester (or carbonate, in the case of bacampicillin) gives an unstable hydroxymethyl ester (8.43) which spontaneously decomposes to ampicillin and either acetaldehyde (bacampacillin) or formaldehyde (pivampicillin). Bacampicillin is a nontoxic prodrug because it decomposes to ampicillin and compounds which are all natural metabolites in the body, namely, CO_2, acetaldehyde, and ethanol (as the usual recommended dose of bacampicillin is 400 mg twice a day, only about 50 μl of ethanol would be released with each dose, so do not expect to get high). Unlike ampicillin, bacampicillin is absorbed to the extent of 98–99%, and ampicillin is liberated into the bloodstream in less than 15 min. Because of the excellent absorption properties of bacampicillin, only one-half to one-third of the ampicillin dose is required orally.

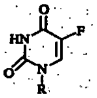


8.41



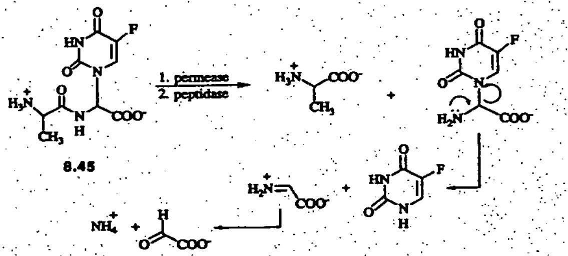
Scheme 8.8. Tripartate prodrugs of ampicillin.

The antitumor agent 5-fluorouracil (**8.44**, R = H) has also been used in the treatment of certain skin diseases. However, because of its low lipophilicity, it does not produce optimal topical bioavailability. *N*-1-Acyloxymethyl derivatives (**8.44**, R = CH_2OCOR') were prepared for increased lipophilicity. The prodrugs were shown to penetrate the skin about 5 times faster than **8.44** (R = H) and to be metabolized to **8.44** (R = H) rapidly.[51] The mechanism for conversion of **8.44** (R = CH_2OCOR') to **8.44** (R = H) is the same as that shown in Scheme 8.8 for ampicillin derivatives.



8.44

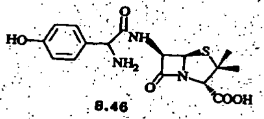
Microorganisms have specialized transport systems for the uptake of peptides (*permeases*), and these transport systems generally have little side-chain specificity. Consequently, peptidyl derivatives of 5-fluorouracil (**8.45**) were designed as potential antifungal and antibacterial agents that would be substrates for both microbial permeases and peptidases.[52] In accord with the known stereochemical selectivity of peptide permeases, only the peptidyl prodrug with the L,L-configuration was active. The mechanism for release of 5-fluorouracil after peptidase action is shown in Scheme 8.9.



Scheme 8.9. Activation of peptidyl derivatives of 5-fluorouracil.

5. Mutual Prodrugs

When it is necessary for two synergistic drugs to be at the same site at the same time, a mutual prodrug approach should be considered. A *mutual prodrug* is a bipartate or tripartate prodrug in which the carrier is a synergistic drug of the drug to which it is linked. In Chapter 5 (Sections V,B,2,a and V,C,3,g) a form of resistance to β-lactam antibacterial drugs was discussed in which these bacteria have a high concentration of the enzyme β-lactamase. For resistant bacteria, compounds that inhibit β-lactamase are given in combination with a β-lactam antibacterial drug. For example, the combination of the penicillin derivative amoxicillin (**8.46**) and the β-lactamase inactivator potassium clavulanate (**8.47**) is used for oral treatment of infections caused by β-lactamase–producing bacteria. Another combination used is the ampicillin prodrug pivampicillin (**8.42**, R = H; R′ = *tert*-Bu) plus the double ester (**8.48**, R = $CH_2OCOCMe_3$) of the β-lactamase inactivator penicillanic acid sulfone (**8.48**, R = H). However, if the two prodrugs are given separately, it is not clear that they are absorbed and transported to the site of action at the same time and in equivalent amounts. An example of a tripartate mutual prodrug is sultamacillin (**8.49**), which upon hydrolysis by an esterase produces ampicillin, penicillanic acid sulfone, and formaldehyde in a reaction like that shown in Scheme 8.8.[53] A mutual prodrug would have a high probability of success provided it is well absorbed, both components are released concomitantly and quantitatively after absorption, the maximal effect of the combination of the two drugs occurs at a 1:1 ratio, and the distribution and elimination of the two components are similar.



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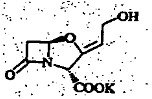

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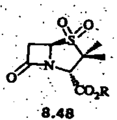

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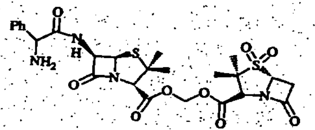


8.49

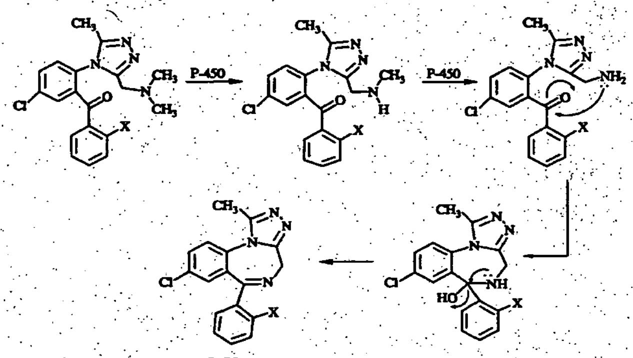
B. Bioprecursor Prodrugs

1. Origins

The birth of bioprecursor prodrugs occurred when it was demonstrated that the antibacterial agent prontosil was active *in vivo* only because it was metabolized to the actual drug sulfanilamide (see Section IV,B,1 of Chapter 5). In this case the azo prodrug prontosil was reduced to the amine sulfa drug. This exemplifies the bioprecursor strategy. Whereas carrier-linked prodrugs rely largely on hydrolysis reactions for their effectiveness, bioprecursor prodrugs mostly utilize either oxidative or reductive activation reactions. The examples given below are arranged according to the type of metabolic activation reaction involved.

2. Oxidative Activation

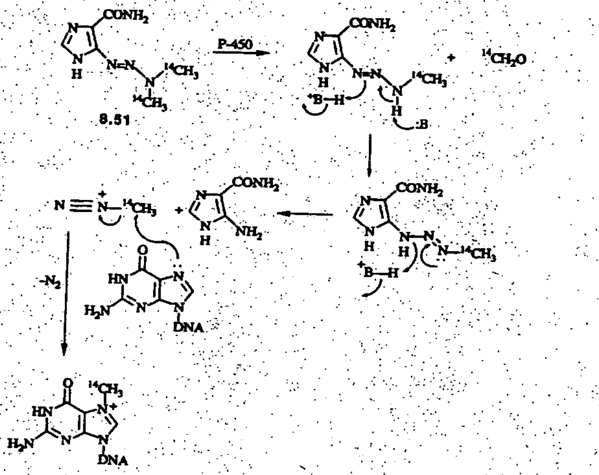
a. N- and O-Dealkylations. Open-ring analogs of benzodiazepines, such as the anxiolytic drug alprazolam (**8.50**, X = H) and the sedative triazolam (**8.50**, X = Cl), undergo metabolic N-dealkylation and spontaneous cyclization (Scheme 8.10).[54]



8.50

Scheme 8.10. Bioprecursor prodrugs for alprazolam and triazolam.

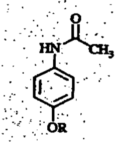
The triazene antitumor drugs are also activated by N-dealkylation.[55] One important class of analogs is the triazenoimidazoles, such as 5-(3,3-dimethyl-1-triazenyl)-1*H*-imidazole-4-carboxamide (**8.51**, dacarbazine) which is active against a broad range of cancers but is used preferably for the treatment of melanotic melanoma.[56] Although dacarbazine is a structural analog of 5-aminoimidazole-4-carboxamide, an intermediate in purine biosynthesis, the cytotoxicity of **8.51** is a result of its conversion to an alkylating agent, not its structural similarity to the metabolic intermediate. With the use of [*methyl*-^{14}C]dacarbazine, it was shown that formaldehyde is generated and that the DNA becomes methylated at the 7 position of guanine.[57] A mechanism that rationalizes these results is shown in Scheme 8.11.



8.51

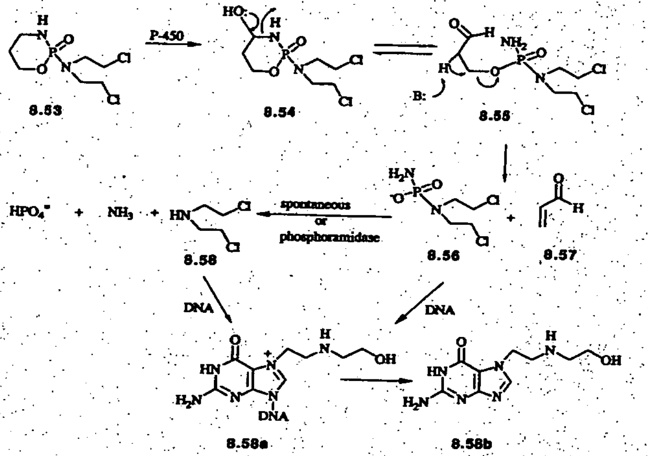
Scheme 8.11. Methylation of DNA by dacarbazine.

An example of a bioprecursor prodrug that is activated by O-dealkylation is the analgesic and antipyretic agent phenacetin (**8.52**, R = CH_2CH_3). Phenacetin owes its activity to its conversion by O-dealkylative metabolism to acetaminophen (**8.52**, R = H).[58]



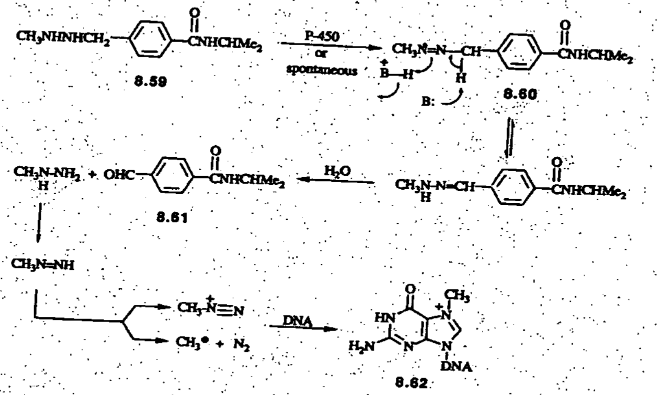
8.52

b. Oxidative Deamination. Because of the high concentration of phosphoramidases in neoplastic cells, hundreds of phosphamide analogs of nitrogen mustards were synthesized and tested as carrier-linked antitumor prodrugs. Cyclophosphamide (**8.53**, Scheme 8.12) emerged as an important drug for the treatment of a wide variety of malignant diseases; however, it was later found that it was inactive in tissue culture. Preincubation of the compound with liver homogenates activated it, suggesting that cyclophosphamide is a prodrug requiring an oxidative mechanism (see Section IV,B,1 of Chapter 7).[59a–c] The activation mechanism is believed to be that shown in Scheme 8.12 (there are other metabolites that are not shown in Scheme 8.12 derived from each of the intermediates). It is not clear which of the toxic metabolites, the phosphoramide mustard (**8.56**) or the parent nitrogen mustard (**8.58**), is responsible for the therapeutic action; the major adduct isolated by high-performance liquid chromatography (HPLC) from *in vitro* and *in vivo* studies in rat is *N*-(2-hydroxyethyl)-*N*-[2-(7-guaninyl)ethyl]amine (**8.58b**),[59d] the hydrolysis product of **8.58a**. The reaction of nitrogen mustards with DNA was discussed in Chapter 6 (Section III,B,1). Acrolein (**8.57**) is a potent Michael acceptor that may be responsible for the hemorrhagic cystitis side effect; administration of sulfhydryl compounds, which react readily with acrolein, can prevent this side effect. Aldehyde dehydrogenase catalyzes the oxidation of **8.54** to the corresponding cyclic amide and the oxidation of **8.55** to the corresponding carboxylic acid; however, both of these metabolites are inactive. It has been suggested that these detoxification reactions occur to a greater extent in normal cells than in cancer cells, which may account for the selective toxicity of cyclophosphamide.[60]



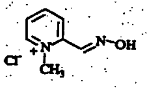
Scheme 8.12. Cytochrome *P*-450–catalyzed activation of cyclophosphamide.

c. N-Oxidation. The antitumor drug used against advanced Hodgkin's disease, procarbazine (**8.59**), is believed to be activated by N-oxidation (Scheme 8.13); it is inert unless treated with liver homogenates or oxidized in neutral solution.[61] Those who have studied Chapter 7 are probably wondering why this circuitous mechanism to **8.61** and methylhydrazine, starting with an N-oxidation reaction, was written instead of a direct conversion of **8.59** to these same metabolites by an oxidative deamination mechanism. The reason is that azoprocarbazine (**8.60**) was identified as the initial metabolic product.[62] 7-Methylguanine (**8.62**) was identified in the urine of mice given procarbazine,[63] which suggests that an activated methylating agent such as methyl diazonium or methyl radical[64] is the reactive intermediate.



Scheme 8.13. Activation of procarbazine.

Another N-oxidation prodrug activation reaction is the reversible redox drug delivery strategy of Bodor and co-workers for getting drugs into the brain[22,23] (see Section II,A,2,c). In the case of pralidoxime chloride (8.63), an antidote for poisoning by organophosphorus pesticides and nerve toxins, the oxidation reaction converts the prodrug to the drug as well as prevents efflux of the drug from the brain. The neurotoxic organophosphorus compounds exert their effects by reacting with acetylcholinesterase, the enzyme found in nervous tissue of all species of animals that catalyzes the hydrolysis of the neurotransmitter acetylcholine after it has served its neurohumoral transmission function. The active site of the enzyme is believed to contain two important binding sites, the anionic site that binds the quaternary ammonium cation of acetylcholine and the ester site where the catalytic hydrolysis of the acetyl group occurs (Scheme 8.14).[65]



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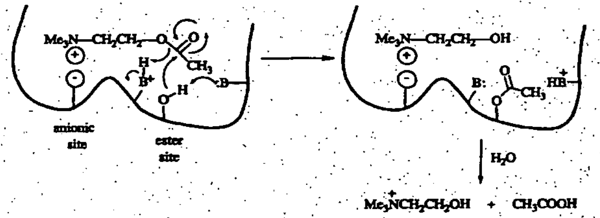
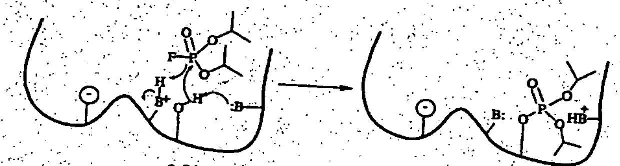


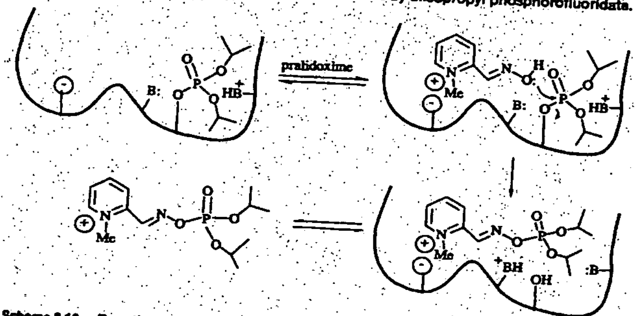
Scheme 8.14. Acetylcholinesterase-catalyzed hydrolysis of acetylcholine.

Organophosphorus compounds, such as the nerve poison diisopropyl phosphorofluoridate (8.64), phosphorylate acetylcholinesterase at the ester site[66] (Scheme 8.15). It was thought that a nucleophilic agent may be capable of dephosphorylating the ester site and reactivating the enzyme. Hydroxylamine appeared to be effective but also was quite toxic. Because acetylcholinesterase has an anionic binding site, quaternary amine analogs were designed, and 2-formyl-1-methylpyridinium chloride oxime (pralidoxime chloride, 8.63) was found to be an effective reactivator of the enzyme (Scheme 8.16). However, 8.63 is very poorly soluble in lipids, so its generation is most likely restricted to the peripheral nervous system; little reactivation of brain acetylcholinesterase was observed *in vivo*.[67] Apparently, the effectiveness of 8.63 as an antidote for organophosphorus nerve poisons results from the fact that the primary damage done by these poisons is to the peripheral nervous system. To improve the permeability of 8.63 into the central nervous system Bodor and co-workers[22,68] prepared the 5,6-dihydropyridine analog 8.65. As 8.65 is uncharged, its permeability through the blood–brain barrier was quite good. Once inside the brain it was oxidized to 8.63.

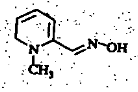


8.64

Scheme 8.15. Phosphorylation of acetylcholinesterase by diisopropyl phosphorofluoridate.

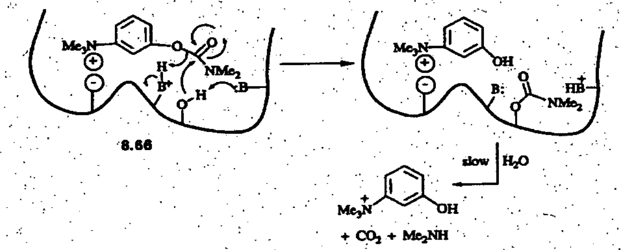


Scheme 8.16. Reactivation of phosphorylated acetylcholinesterase by pralidoxime chloride.



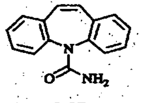
8.65

It is interesting to note that whereas irreversible inactivators of acetylcholinesterase, such as organophosphorus nerve gases, are highly toxic, compounds that form weakly stable covalent bonds to the serine residue in the ester binding site are useful therapeutic agents. This inhibition of acetylcholinesterase results in the enhancement of cholinergic action by facilitating the transmission of impulses across neuromuscular junctions, which has a cholinomimetic effect on skeletal muscle. An example of this is neostigmine (**8.66**), a drug used in the treatment of the neuromuscular disease myasthenia gravis. Neostigmine carbamylates the active site serine residue of acetylcholinesterase; the carbamate, however, hydrolyzes slowly so that, in effect, **8.66** acts as a reversible inhibitor of the enzyme (Scheme 8.17). Therefore, the difference in effects of the acetylcholinesterase substrates and inhibitors is derived from the stabilities of the covalent adducts. The acetylated serine formed from acetylcholine (a substrate) hydrolyzes readily, the carbamylated serine produced from neostigmine (an inhibitor) hydrolyzes slowly, and the phosphorylated serine from organophosphorus compounds (inactivators) is stable to hydrolysis.



Scheme 8.17. Carbamylation of acetylcholinesterase by neostigmine.

d. Other Oxidations. Carbamazepine (**8.67**) is an anticonvulsant drug that is the metabolic precursor of the active agent, carbamazepine 10,11-oxide (**8.68**).[69] Cysteine conjugates of the antitumor agent elliptinium acetate (**8.69**) found in the urine of patients taking the drug indicate that an oxidative mechanism is important (Scheme 8.18) and suggest that alkylation, as well as intercalation, of DNA by **8.69** may be a viable mechanism of action.[70]



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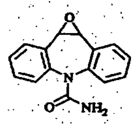

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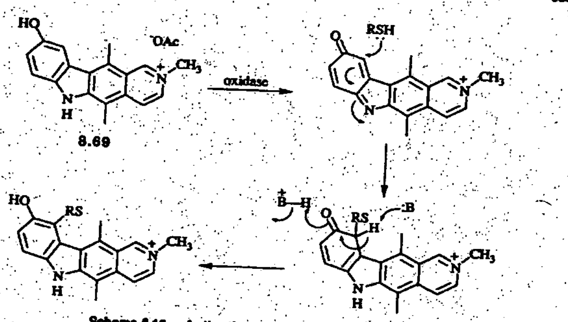
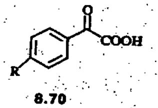


Scheme 8.18. Activation of elliptinium acetate.

Stimulation of pyruvate dehydrogenase results in a change of myocardial metabolism from fatty acid to glucose utilization. Because the latter requires less oxygen consumption, glucose utilization is beneficial to patients with ischemic heart disease in which arterial blood flow is blocked and therefore less oxygen is available.[71] Arylglyoxylic acids (**8.70**) are important stimulators of this enzyme, but they have short durations of action. L-(+)-2-(4-Hydroxyphenyl)glycine (oxfenicine; **8.71**, R = OH) is a stable amino acid that is actively transported across lipid membranes and is rapidly transaminated (see Section III,A,3 of Chapter 4) to 4-hydroxyphenylglyoxylic acid (**8.70**, R = OH).[72] This active transport system and rapid conversion of the prodrug to the drug allow a higher concentration of the active drug to persist at the desired site of action longer.



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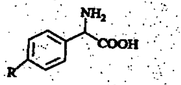
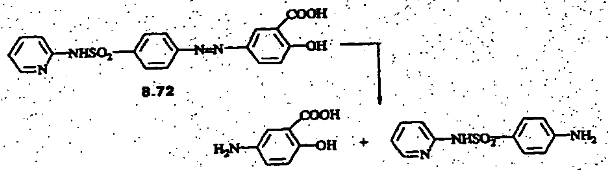


8.71

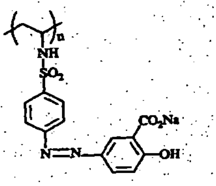
3. Reductive Activation

a. Azo Reduction. As described in Section II,B,1 the paradigm for bioprecursor prodrugs, prontosil, is activated by reduction of its azo linkage to the true bacteriostatic agent, sulfanilamide.

Sulfasalazine (**8.72**), which is used in the treatment of inflammatory bowel disease (ulcerative colitis), is reductively cleaved by anaerobic bacteria in the lower bowel to 5-aminosalicylic acid (**8.73**) and sulfapyridine (**8.74**); **8.73** is the therapeutic agent, and **8.74** produces adverse side effects (Scheme 8.19).[73] A macromolecular drug delivery system was developed to improve the therapeutic index of this drug. The drug (8.73) was azo-linked at the 5 position through a spacer to poly(vinyl amine) (8.75).[74] The advantages of this polymeric drug delivery system are that it is not absorbed or metabolized in the small intestine, 8.73 can be released by reduction at the disease site, and the carrier polymer is not absorbed or metabolized. The water-soluble polymer-linked drug (8.75) was more active than 8.72 or 8.73 in the guinea pig ulcerative colitis model.

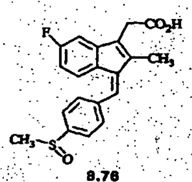


Scheme 8.19. Reductive activation of sulfasalazine.



8.75

b. Sulfoxide Reduction. The antiarthritis drug sulindac (8.76) is an indene isostere (see Section II,D,4 of Chapter 2) of the nonsteroidal anti-inflammatory (antiarthritis) drug indomethacin (8.77), which originally was designed as a serotonin analog. Sulindac is less irritating to the gastrointestinal tract and produces many fewer and more mild central nervous system effects than does indomethacin.[75] The 5-fluoro group was substituted for the methoxyl group to improve the analgesic properties, and the *p*-methylsulfinyl group was substituted for the chlorine atom to increase the solubility. Sulindac is inactive *in vitro* but is highly active *in vivo*. The corresponding sulfide, however, is active *in vitro* and *in vivo*. Therefore, sulindac is a prodrug for the sulfide, the metabolic reduction product.



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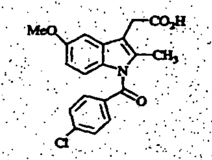
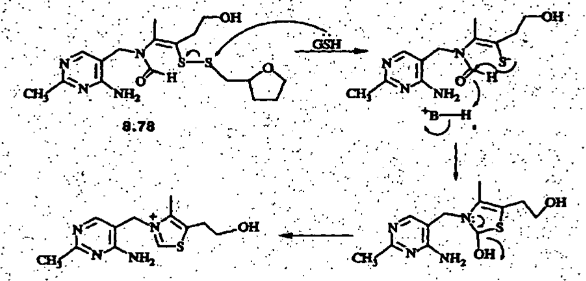


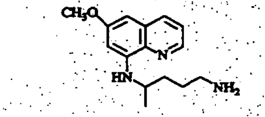
8.77

c. Disulfide Reduction. Because thiamin (vitamin B_1; 8.79) is a quaternary ammonium salt, it is poorly absorbed into the central nervous system and from the gastrointestinal tract. To increase the lipophilicity thiamin tetrahydrofurfuryl disulfide (8.78, Scheme 8.20) was designed as a lipid-soluble prodrug of thiamin.[76] The prodrug permeates rapidly through red blood cell membranes (as a model for other membranes) and reacts with glutathione to produce thiamin.[77]



Scheme 8.20. Conversion of thiamin tetrahydrofurfuryl disulfide to thiamin.

To diminish the toxicity of the antimalarial drug primaquine (8.80) and target it for cells that contain the malaria parasite, a macromolecular drug delivery system was designed[78] (8.81). The lactose-linked albumin was used for improved uptake in the liver via the asialoglycoprotein receptor system. Because the concentration of free thiol in the blood is relatively low, but is high intracellularly, it was expected that thiol reduction of the disulfide linkage would occur mostly inside the cell. It is not known if after disulfide reduction the cysteinyl residue is detached by hydrolysis or remains attached to primaquine. The therapeutic index of 8.81, however, was 12 times higher than that of the free drug in *Plasmodium*-infected mice.



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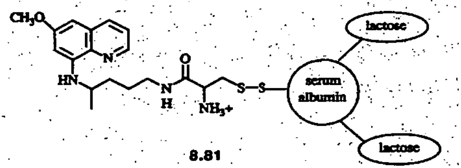
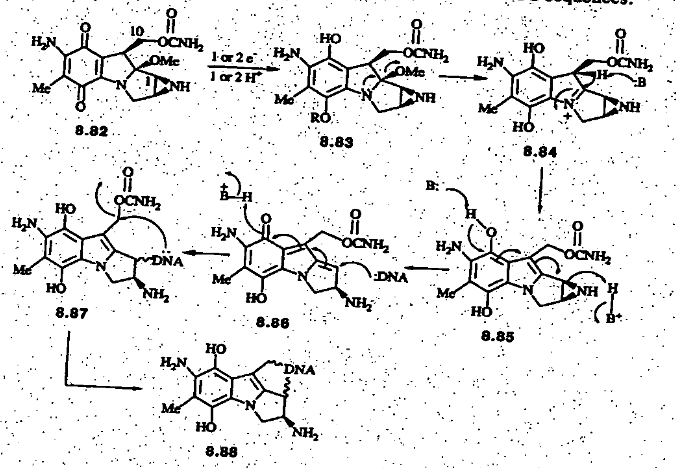


8.81

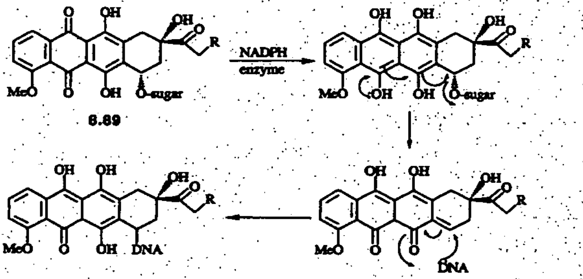
d. Bioreductive Alkylation. *Bioreductive alkylation* is a prodrug strategy in which an inactive compound is metabolically reduced to an alkylating agent.[79] The prototype for antitumor antibiotics that act as bioreductive alkylating agents of DNA is mitomycin C (**8.82**, Scheme 8.21) which contains three important carcinostatic functional groups, the quinone, the aziridine, and the carbamate group.[79-81] The mechanism proposed by Iyer and Szybalski[80] as modified by Moore and Czerniak[79] is shown in Scheme 8.21. Reduction of the quinone by one electron to the semiquinone (**8.83**, R = electron) or by two electrons to the hydroquinone (**8.83**, R = H) converts the heterocyclic nitrogen from a vinylogous amide nitrogen (the nonbonded electrons of the nitrogen are in conjugation with the quinone carbonyl via the intermediate double bond), which is not nucleophilic, to an amine nitrogen, which can eliminate the β-methoxide ion (**8.83**). Tautomerization of the resultant immonium ion (**8.84**) gives **8.85**, which is set up for aziridine ring opening. This activates the drug by unmasking the electrophilic site at C-1 which alkylates the DNA (**8.86**). A subsequent reaction of DNA at C-10 (**8.87**) results in the cross-linking of the DNA (**8.88**). Bean and Kohn[82] showed in chemical models that nucleophiles react most rapidly at C-1; the reaction at C-10 to displace the carbamate also occurs, but at a slower rate. Reduction of the quinone is necessary for the covalent reaction of **8.82** to DNA, but controversy exists as to whether the semiquinone (**8.83**, R = electron) or hydroquinone (**8.83**, R = H) is the viable intermediate.[81] Chemical model studies on the mechanism of action of mitomycin C indicate that the conversion of **8.82** to **8.87** can occur at the semiquinone stage[83] and the conversion of **8.87** to **8.88** occurs at the hydroquinone oxidation state.[84] Both monoalkylated and bis-alkylated DNA adducts have been identified; the extent of mono- and bis-alkylation increases with increasing guanine base composition of the DNA.[85] The site of attachment of these adducts is at N-2 of the guanine bases[86a,b] with preferential interstrand cross-linking at 5′-CG rather than 5′-GC sequences.[86c]



Scheme 8.21. Bioactivation of mitomycin C.

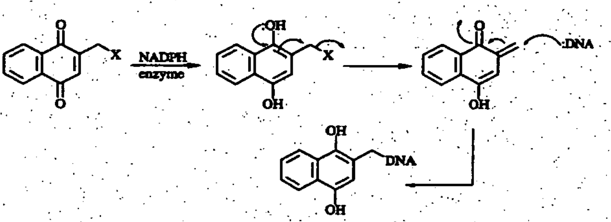
Several other naturally occurring antitumor quinones may be involved in this type of mechanism.[79] Anthracycline antitumor agents such as doxorubicin (**8.89**, R = OH) and daunorubicin (**8.89**, R = H) were suggested to act as one-electron bioreductive alkylating agents[87a] (Scheme 8.22); however, the hydroquinone has been synthesized, and it does not eliminate the sugar.[87b] Therefore, if the quinone methide mechanism is relevant to the anthracycline antibiotics, elimination must occur at the semiquinone oxidation state. Radical-induced reactions of anthracycline antitumor antibiotics were discussed in Section III,C,1 of Chapter 6.

The bioreductive alkylation approach was directed toward the design of new antineoplastic agents that may be selective for hypoxic (O_2-deficient) cells in solid tumors.[88] These cells are remote from blood vessels and are located at the center of the solid tumors. Hypoxia protects the tumor cells from radiation therapy, and because these cells are buried deep inside the



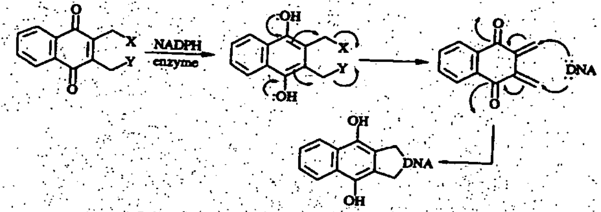
Scheme 8.22. Anthracycline antitumor agents as bioreductive alkylators.

tumor, appropriate concentrations of antitumor drugs may not reach them prior to drug metabolism. As these cells might have a more efficient reducing environment, bioreductive alkylation seemed to be well suited. The bioreductive alkylation approach based on reduction of a quinone to the corresponding hydroquinone was utilized in the design of the prodrugs. Both mono- (Scheme 8.23)[89] and bisalkylating agents (Scheme 8.24)[90] were developed. Electron-rich substituents lower the reduction potential of the quinones and make them more reactive.[91]

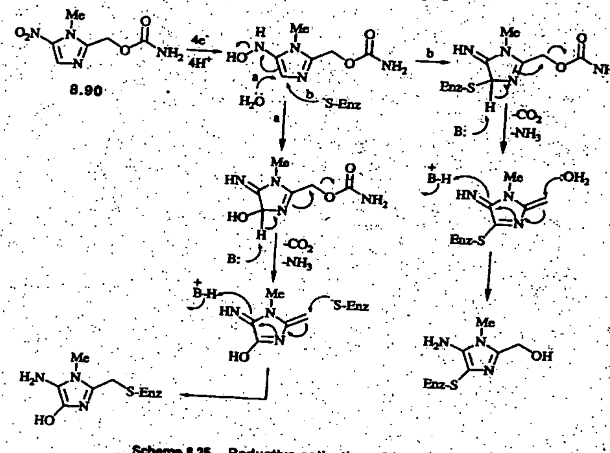


Scheme 8.23. Bioreductive monoalkylating agents.

e. Nitro Reduction. The mechanism of action of the antiprotozoal agent ronidazole (8.90) is not known, but on the basis of metabolism studies using several radioactively labeled analogs, it was suggested that 8.90 is activated by initial four-electron reduction of the 5-nitro group to the corresponding hydroxylamine which can react with protein thiols by one of two mechanisms (Scheme 8.25).[92]



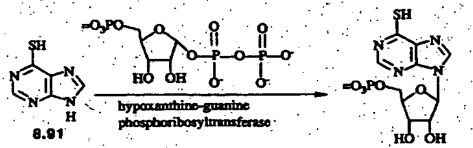
Scheme 8.24. Bioreductive bisalkylating agents.



Scheme 8.25. Reductive activation of ronidazole.

4. Nucleotide Activation

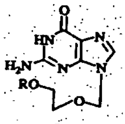
The antineoplastic agent 6-mercaptopurine (8.91) produces a 50% remission rate for acute childhood leukemias. Although 8.91 inhibits several enzyme systems, these inhibitions are irrelevant to its anticancer activity. Only tumors that convert the drug to its nucleotide are affected. 6-Mercaptopurine is activated by a reaction with 5-phosphoribosylpyrophosphate, catalyzed by hypoxanthine–guanine phosphoribosyltransferase (hypoxanthine phosphoribosyltransferase) (Scheme 8.26). The nucleotide (**8.92**) inhibits several enzymes in the purine nucleotide biosynthetic pathway, but the most prominent site is one of the early enzymes in the *de novo* pathway, namely, phosphoribosylpyrophosphate amidotransferase (amidophosphoribosyltransferase), which catalyzes the conversion of phosphoribosylpyrophosphate to phosphoribosylamine.[93] 5-Fluorouracil (see Section V,C,3,e of Chapter 5) is similar to 6-mercaptopurine in the sense that it must first be converted to the corresponding deoxyribonucleotide in order for it to be active.



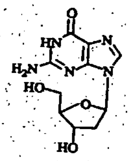
Scheme 8.26. Nucleotide activation of 6-mercaptopurine.

5. Phosphorylation Activation

The antiviral drug acyclovir (**8.93**, R = H) is highly effective against genital herpes simplex virus and varicella zoster virus infections. Its structure can be drawn so that it closely resembles the structure of 2′-deoxyguanosine (**8.94**). Acyclovir itself is inactive, but it is selectively phosphorylated by a viral thymidine kinase to the corresponding monophosphate (**8.93**, R = PO_3^{2-}).[94] Uninfected cells do not phosphorylate acyclovir, and this accounts for the selective toxicity of acyclovir toward viral cells. The second step in the activation of acyclovir is the conversion of the monophosphate (**8.93**, R = PO_3^{2-}) to the diphosphate (**8.93**, R = $P_2O_6^{3-}$), catalyzed by guanylate kinase.[95] The final activation step is the conversion of the diphosphate to the triphosphate (**8.93**, R = $P_3O_9^{4-}$), which could be accomplished by a variety of enzymes, particularly phosphoglycerate kinase.[96] Further selective toxicity is derived from the fact that acyclovir triphosphate is selectively taken up by viral α-DNA polymerases as its structure resembles that of the essential DNA precursor, deoxyguanosine triphosphate. The K_i for viral α-DNA polymerase is up to 40 times lower than that for normal cellular α-DNA polymerase.[97] Acyclovir triphosphate is a substrate for the viral polymerase but not for the normal cellular polymerase; however, incorporation of acyclovir triphosphate into the viral DNA leads to the formation of a *dead-end complex* (an enzyme–substrate complex that is no longer active) after the next deoxynucleotide triphosphate unit is incorporated.[98] This disrupts the replication cycle of the virus and destroys it. Even if the phosphorylated acyclovir were released from the virus cell, it would be too polar to be taken up by normal cells, and, as indicated above, the triphosphate is a poor substrate for normal human α-DNA polymerase anyway. Therefore, this drug exhibits a high degree of selective toxicity against viral cells.



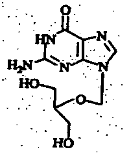
8.93



8.94

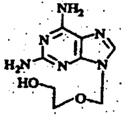
As might be predicted from knowledge of the mechanism of acyclovir, acquired resistance to the drug can occur by three different mechanisms. Because of the importance of the thymidine kinase to the activation of acyclovir, resistance could arise from a deletion of this enzyme[99] or a change in its substrate specificity.[100] The third mechanism could be an altered viral DNA polymerase.[99]

Ganciclovir (**8.95**) is an analog of acyclovir that has a conformation resembling the structure of 2′-deoxyguanosine even closer than does acyclovir. This compound is about as active as acyclovir against herpes simplex viruses and varicella zoster virus but is much more inhibitory than acyclovir against human cytomegalovirus,[101] an important pathogen in immunocompromised and acquired immune deficiency syndrome (AIDS) patients.

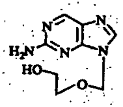


8.95

Only 15–20% of acyclovir is absorbed after oral administration. Consequently, two prodrugs for acyclovir have been designed to improve gastrointestinal absorption and to protect acyclovir against biotransformations to inactive metabolites. 2,6-Diamino-9-(2-hydroxyethoxymethyl)purine (**8.96**) is converted to acyclovir by the enzyme adenosine deaminase[102] (catalyzes the hydrolysis of adenosine to inosine), and 6-amino-9-(2-hydroxyethoxymethyl)purine (8.97, 6-deoxyacyclovir) is oxidized to acyclovir by xanthine oxidase.[103] The latter compound is 18 times more water soluble than acyclovir. In humans urinary excretion of acyclovir is 5–6 times greater when 8.97 is given than an equivalent dose of acyclovir.



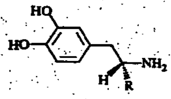
8.96



8.97

6. Decarboxylation Activation

The striatal tracts, which are important for the control of voluntary movements, contain a balance of the inhibitory neurotransmitter dopamine and the excitatory neurotransmitter acetylcholine. An imbalance in the dopaminergic and cholinergic components produces disorders of movement. In Parkinson's disease there is a marked deficiency in the dopaminergic component which is attributed to the loss of dopaminergic neurons in the substantia nigra. The obvious treatment for Parkinson's disease would be to give high doses of dopamine (8.98, R = H), but this does not work because dopamine does not cross the blood–brain barrier. However, there is an active transport system for L-amino acids; consequently, L-dopa (8.98, R = COOH) is transported into the brain where it is decarboxylated by the pyridoxal 5'-phosphate–dependent enzyme (see Section III,A,2 of Chapter 4) aromatic-L-amino acid decarboxylase (also called dopa decarboxylase) to dopamine. Since the D,L-mixture produces unwanted side effects, levodopa (L-dopa) is used as a prodrug for dopamine. Unfortunately, because dopaminergic neurons cannot be rejuvenated, levodopa does not reverse the course of the disease, it merely halts (actually only slows) its progression.[104]

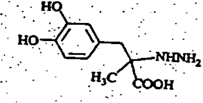


8.98

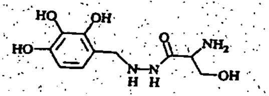
As discussed in Section V,C,3,d of Chapter 5, dopamine is a substrate for monoamine oxidase B; consequently, as levodopa is being converted to dopamine in the brain, monoamine oxidase B is degrading the dopamine. An inactivator of monoamine oxidase B, L-deprenyl, is now used in combination

with levodopa to minimize the degradation of the dopamine generated by levodopa.[105]

One major complication with the use of levodopa therapy arises from the fact that aromatic-L-amino acid decarboxylase also exists in the periphery (outside of the central nervous system), and greater than 95% of the orally administered levodopa is decarboxylated in its first pass through the liver and kidneys. Possibly only 1% of the levodopa taken actually penetrates into the central nervous system. If the peripheral aromatic-L-amino acid decarboxylase could be inhibited without inhibition of the same enzyme in the brain, the levodopa would be protected from the undesired metabolism. This, in fact, is possible because inhibitors of aromatic-L-amino acid decarboxylase are charged molecules, and unless they are actively transported, they will not cross the blood–brain barrier. Carbidopa (8.99) is used in the United States, and benserazide (8.100) is used in Europe and Canada in combination with levodopa for the treatment of Parkinson's disease. With the combined use of a peripheral aromatic-L-amino acid decarboxylase inhibitor, the optimal effective dose of levodopa can be reduced by greater than 75%.



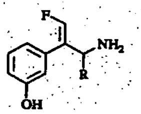
8.99



8.100

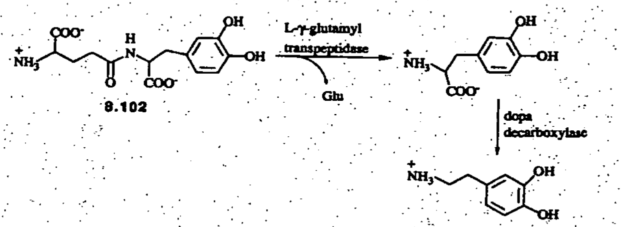
In Section V,C,3,c of Chapter 5, the application of inactivators of monoamine oxidase A (MAO A) as antidepressant agents was discussed. Although MAO inactivators are used in the treatment of depression, a severe cardiovascular side effect can result unless the diet is controlled to minimize the intake of tyramine-containing foods. This side effect results from the concurrent inactivation of the peripheral MAO A along with the brain MAO A. A brain-specific MAO A inactivator would give the desired antidepressant effect without the undesirable cardiovascular effect. A prodrug approach for the brain-selective delivery of a MAO A–selective inactivator was developed at Merrell Dow.[106,107] This particular type of prodrug was termed a *dual enzyme-activated inhibitor*[107] because the activating enzyme is, by design, part of the same metabolic pathway as the enzyme that is targeted for inhibition. In this case the activating enzyme is aromatic-L-amino acid decarboxylase and the target enzyme is MAO A. (*E*)-β-Fluoromethylene-*m*-tyramine (8.101, R = H) is a mechanism-based inactivator (see Section V,C of Chapter 5) of monoamine oxidase with selectivity for MAO A.[108] The corresponding amino acid, (*E*)-β-fluoromethylene-*m*-tyrosine (8.101, R = COOH)[107] is not an inhibitor of MAO, but it is a good substrate for aromatic-L-amino acid decarboxylase, which converts 8.101 (R = COOH) to 8.101 (R = H). The amino acid

(**8.101**, R = COOH) is actively transported into the central nervous system and is concentrated in the synaptosomes. Because brain aromatic-L-amino acid decarboxylase is located predominantly in monoamine nerve endings, **8.101** (R = COOH) is decarboxylated to **8.101** (R = H) at the desired site of action. To prevent inactivation of peripheral MAO A, **8.101** (R = COOH) is administered with carbidopa, which blocks peripheral aromatic-L-amino acid decarboxylase–catalyzed decarboxylation of **8.101** (R = COOH). This results in brain-selective MAO A inactivation with little or no peripheral MAO A inhibition and only a minimal tyramine effect.



8.101

Not only is dopamine a major inhibitory neurotransmitter, but it also plays an important role in the kidneys. Dopamine increases systolic and pulse blood pressure and renal blood flow. If it is desired to have selective delivery of dopamine to the kidneys in order to attain renal vasodilation without a blood pressure effect, a prodrug for dopamine can be used. There is a high concentration of L-γ-glutamyltranspeptidase, the enzyme that catalyzes the transfer of the L-glutamyl group from the N-terminus of one peptide to another, in kidney cells. Consequently, an L-γ-glutamyl derivative of an amino acid or amine drug could be cleaved selectively in the kidneys.[109] L-γ-Glutamyl-L-dopa (**8.102**) is selectively accumulated in the kidneys, and the L-dopa released by L-glutamyltranspeptidase (γ-glutamyltransferase) is decarboxylated to dopamine by aromatic-L-amino acid decarboxylase, which also is abundant in kidneys (Scheme 8.27).[110] Even at high concentrations of this compound little central nervous system effect is apparent. This, then, is an example of a site-selective carrier-linked prodrug of a bioprecursor prodrug for dopamine.



Scheme 8.27. Metabolic activation of L-γ-glutyamyl-L-dopa to dopamine.

Drug design is typically initiated with approaches to maximize the pharmacodynamic properties of molecules (increased binding to a receptor). A compound may be found that has the desired *in vitro* properties but has unfavorable *in vivo* properties. It should be apparent, then, from the discussion in this chapter that it may be possible to alter the structure of the compound to improve its pharmacokinetic properties and, thereby, transform it into a promising drug candidate.

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